

Laboratory Exercise 60

Skeletal Muscle Contraction

Materials Needed

Textbook
Recording system (kymograph, Physiograph, etc.)
Stimulator and connecting wires
Live frog
Dissecting tray
Dissecting instruments
Probe for pithing
Heavy thread
Frog Ringer's solution

For Demonstration A—The Kymograph:

Kymograph recording system
Electronic stimulator (or inductorium)
Frog muscle (from pithed frog)
Probe for pithing
Dissecting instruments
Frog Ringer's solution

For Demonstration B—The Physiograph:

Physiograph
Myograph and stand
Frog muscle (from pithed frog)
Probe for pithing
Dissecting instruments
Frog Ringer's solution



Safety

- Wear disposable gloves when handling the frogs.
- Dispose of gloves and frogs as instructed.
- Wash your hands before leaving the laboratory.

To study the characteristics of certain physiological events such as muscle contractions, it often is necessary to use a recording device, such as a *kymograph* or a *Physiograph*. These devices can provide accurate recordings of various physiological changes.

To observe the phenomenon of skeletal muscle contractions, muscles can be removed from anesthetized frogs. These muscles can be attached to recording systems and stimulated by electrical shocks of varying strength, duration, and frequency. Recordings obtained from such procedures can be used to study the basic characteristics of skeletal muscle contractions.

Purpose of the Exercise

To observe and record the responses of an isolated frog muscle to electrical stimulation of varying strength and frequency.

LEARNING OUTCOMES

After completing this exercise, you should be able to

- 1 Match the term with the definition of a muscle response.
- 2 Choose a recording system and stimulator to record frog muscle responses to electrical stimulation.
- 3 Determine the threshold level of electrical stimulation in frog muscle.
- 4 Determine the intensity of stimulation needed for maximal muscle contraction.
- 5 Record a single muscle twitch and identify its phases.
- 6 Record the response of a muscle to increasing frequency of stimulation and identify the patterns of tetanic contraction and fatigue.

Demonstration A—The Kymograph

1. Observe the kymograph and, at the same time, study figure 60.1 to examine the names of its major parts.
2. Note that the kymograph consists of a cylindrical *drum* around which a sheet of paper is wrapped. The drum is mounted on a motor-driven *shaft*, and the speed of the motor can be varied. Thus, the drum can be rotated rapidly if rapid physiological events are being recorded or rotated slowly for events that occur more slowly.

A *stylus* that can mark on the paper is attached to a *movable lever*, and the lever, in turn, is connected to an isolated muscle. The origin of the muscle is fixed in position by a *clamp*, and its insertion is hooked to the muscle lever. The muscle also is connected by wires to an *electronic stimulator*

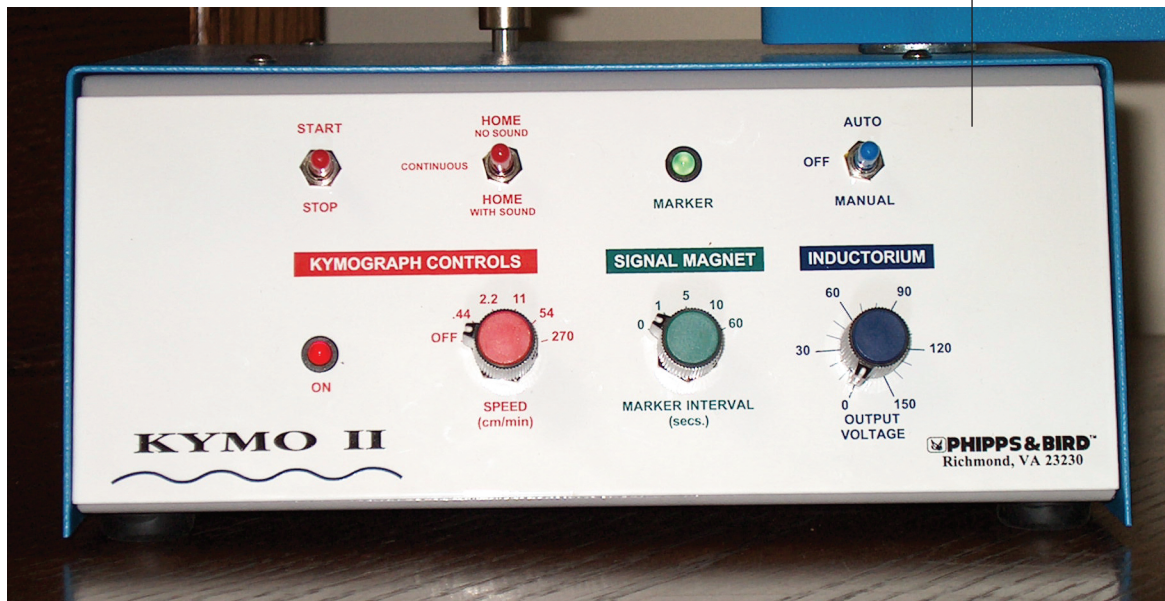
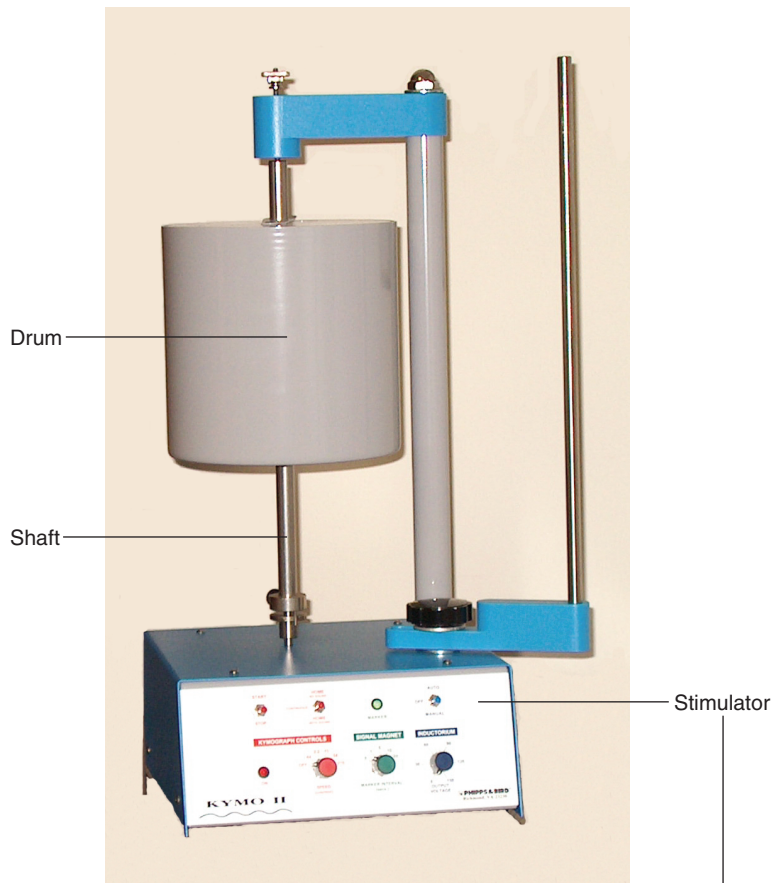


Figure 60.1 Kymograph to record frog muscle contractions.

(or inductorium). The stimulator can deliver single or multiple electrical shocks to the muscle, and it can be adjusted so that the intensity (voltage), duration (milliseconds), and frequency (stimuli per second) can be varied. Another stylus, on the *signal marker* records the time each stimulus is given to

the muscle. As the muscle responds, the duration and relative length of its contraction are recorded by the stylus on the muscle lever.

3. Watch carefully while the laboratory instructor demonstrates the operation of the kymograph to record a frog muscle contraction.

Demonstration B—The Physiograph

1. Observe the Physiograph and, at the same time, study figures 60.2 and 60.3 to examine the names of its major parts.
2. Note that the recording system of the Physiograph includes a transducer, an amplifier, and a recording pen. The *transducer* is a sensing device that can respond to some kind of physiological change by sending an electrical signal to the amplifier. The *amplifier* increases the strength of the electrical signal and relays it to an electric motor that moves the *recording pen*. As the pen moves, a line is drawn on paper.



Figure 60.2 Physiograph to record frog muscle contractions.

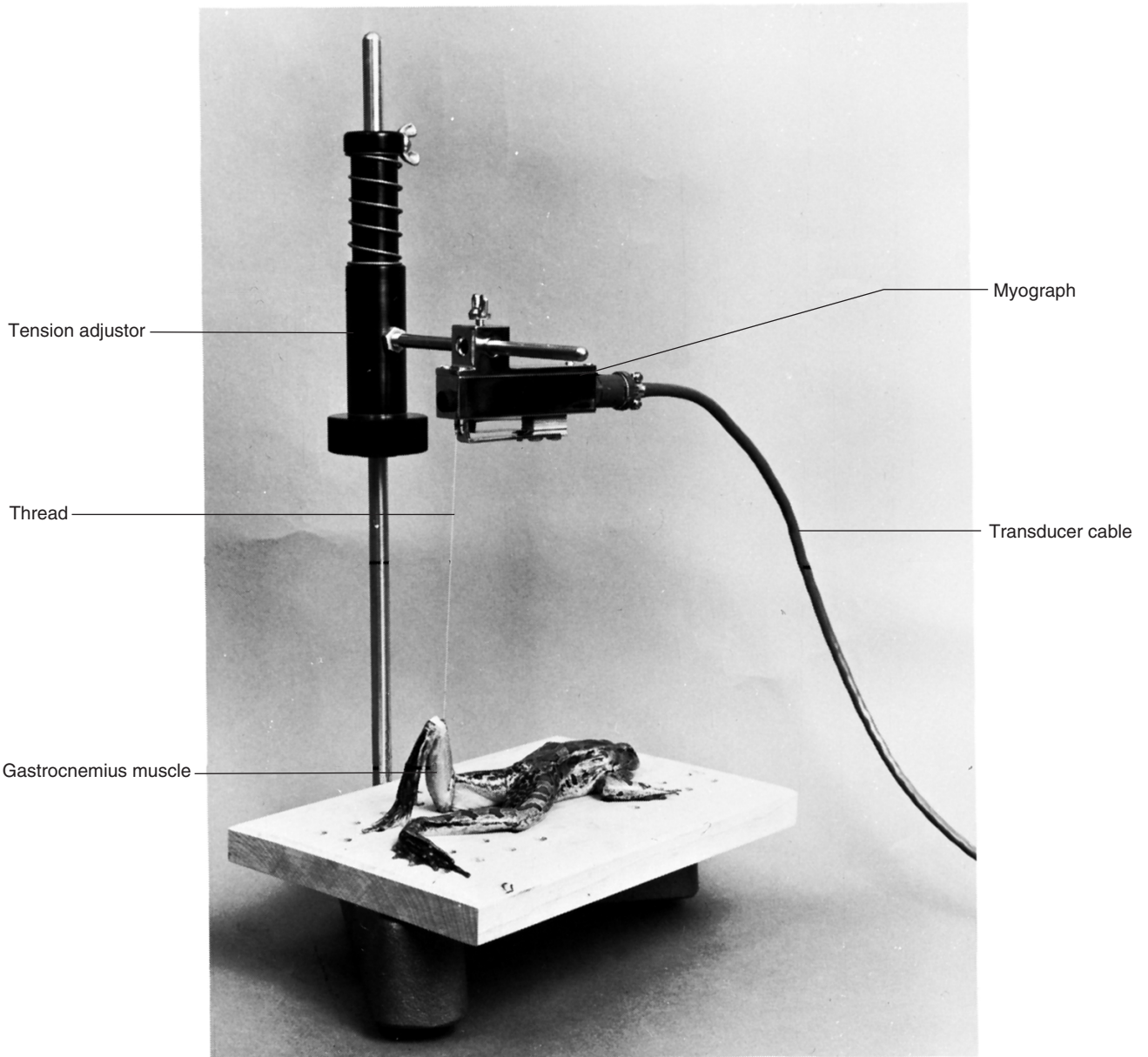


Figure 60.3 Myograph attached to frog muscle.

To record a frog muscle contraction, a transducer called a *myograph* is used (figure 60.3). The origin of the muscle is held in a fixed position, and its insertion is attached to a small lever in the myograph by a thread. The myograph, in turn, is connected to the amplifier by a transducer cable. The muscle also is connected by wires to the electronic stimulator, which is part of the Physiograph. This stimulator can be adjusted to deliver single or multiple electrical shocks to the muscle, and the intensity (voltage), duration (milliseconds), and frequency (stimuli per second) can be varied.

The speed at which the paper moves under the recording pen can be controlled. A second pen, driven by a timer, marks time units on the paper and indicates when the stimulator is activated. As the muscle responds to stimuli, the recording pen records the duration and relative length of each muscle contraction.

3. Watch carefully while the laboratory instructor operates the Physiograph to record a frog muscle contraction.

EXPLORE

Procedure A—Textbook Review

1. Review the section entitled “Muscular Responses” in chapter 9 of the textbook.
2. Complete Part A of Laboratory Report 60.

EXPLORE

Procedure B—Recording System

1. Set up the recording system and stimulator to record the contractions of a frog muscle according to the directions provided by the laboratory instructor.
2. Obtain a live frog, and prepare its calf muscle (gastrocnemius) as described in Procedure C.

EXPLORE

Procedure C—Muscle Preparation

1. Prepare the live frog by pithing so that it will not have sensations or movements when its muscle is removed. To do this, follow these steps:
 - a. Hold the frog securely in one hand so that its limbs are extended downward.
 - b. Position the frog’s head between your thumb and index finger (fig. 60.4a).
 - c. Bend the frog’s head forward at an angle of about 90° by pressing on its snout with your index finger.

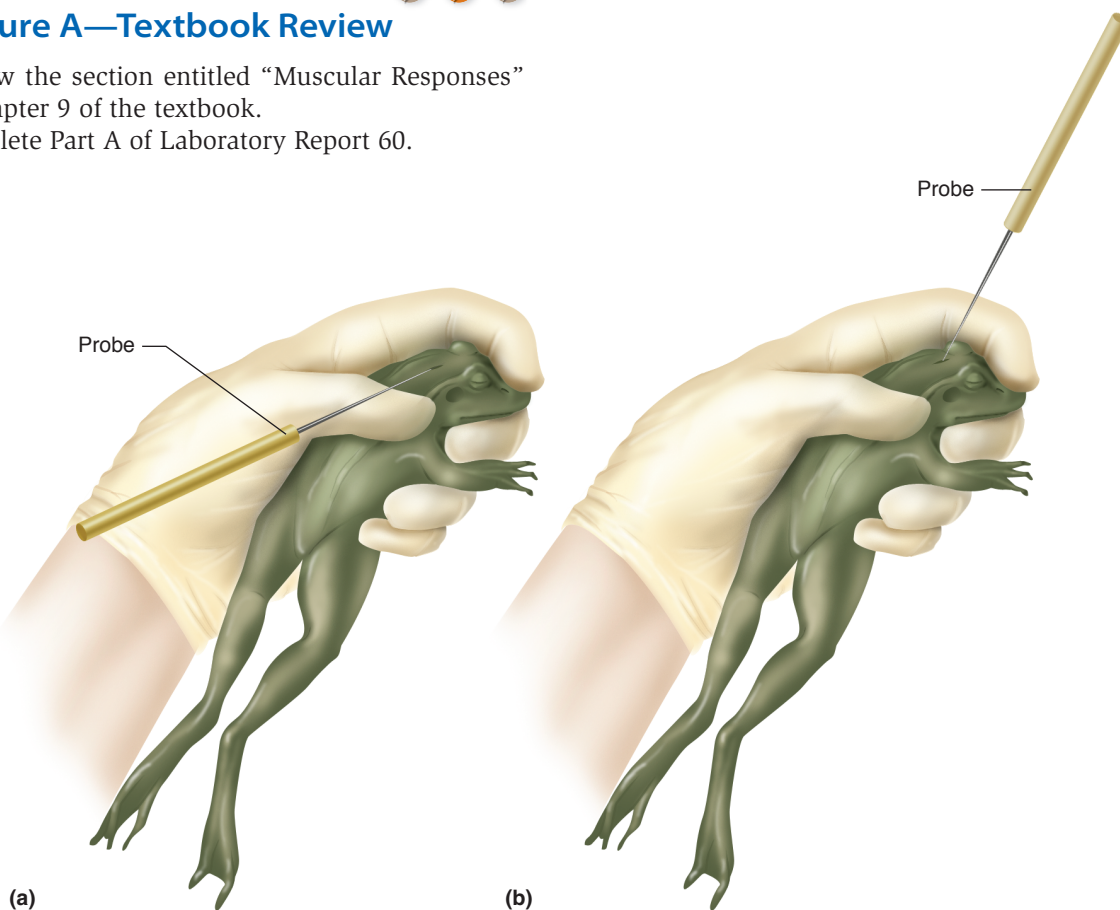


Figure 60.4 Hold the frog’s head between your thumb and index finger to pith (a) its brain and (b) its spinal cord.

- d. Use a sharp probe to locate the foramen magnum between the occipital condyles in the midline between the frog's tympanic membranes.
- e. Insert the probe through the skin and into the foramen magnum, and then quickly move the probe from side to side to separate the brain from the spinal cord.
- f. Slide the probe forward into the braincase, and continue to move the probe from side to side to destroy the brain.
- g. Remove the probe from the braincase, and insert it into the spinal cord through the same opening in the skin (fig. 60.4b).
- h. Move the probe up and down the spinal cord to destroy it. If the frog has been pithed correctly, its legs will be extended and relaxed. Also, the eyes will not respond when touched with a probe.

Alternative Procedure

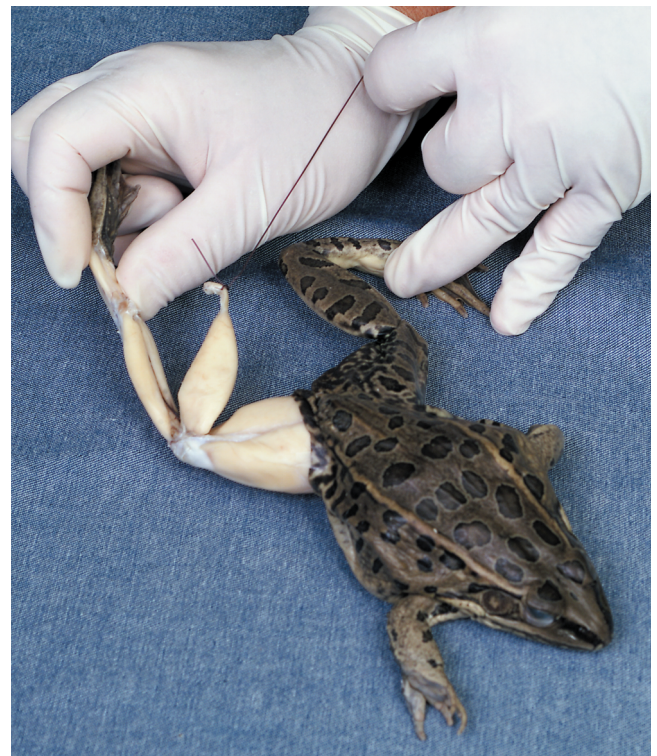
An anesthetizing agent, tricaine methane sulfonate, can be used to prepare frogs for this lab. This procedure eliminates the need to pith frogs.

2. Remove the frog's gastrocnemius muscle by proceeding as follows:
 - a. Place the pithed frog in a dissecting tray.
 - b. Use scissors to cut through the skin completely around the hindlimb in the thigh.
 - c. Pull the skin downward and off the hindlimb.
 - d. Locate the gastrocnemius muscle in the calf and the calcaneal tendon (Achilles tendon) at its distal end (fig. 60.5a).
 - e. Separate the calcaneal tendon from the underlying tissue, using forceps.
 - f. Tie a thread firmly around the tendon (fig. 60.5b).
 - g. When the thread is secure, free the distal end of the tendon by cutting it with scissors.
 - h. Attach the frog muscle to the recording system in the manner suggested by your laboratory instructor (see figs. 60.1 and 60.3).
 - i. Insert the ends of the stimulator wires into the muscle so that one wire is located on either side of the belly of the muscle.

Keep the frog muscle moist at all times by dripping frog Ringer's solution on it. When the muscle is not being used, cover it with some



(a)



(b)

Figure 60.5 (a) Separate the calcaneal (Achilles) tendon from the underlying tissue. (b) Tie a thread around the tendon, and cut its distal attachments.

paper toweling that has been saturated with frog Ringer's solution.

Before you begin operating the recording system and stimulator, have the laboratory instructor inspect your setup.

EXPLORE

Procedure D—Threshold Stimulation

1. To determine the threshold or minimal strength of electrical stimulation (voltage) needed to elicit a contraction in the frog muscle, follow these steps:
 - a. Set the stimulus duration to a minimum (about 0.1 milliseconds).
 - b. Set the voltage to a minimum (about 0.1 volts).
 - c. Set the stimulator so that it will administer single stimuli.
2. Administer a single stimulus to the muscle and watch to see if it responds. If no response is observed, increase the voltage to the next higher setting and repeat the procedure until the muscle responds by contracting.
3. After determining the threshold level of stimulation, continue to increase the voltage in increments of 1 or 2 volts until a maximal muscle contraction is obtained.
4. Complete Part A of the laboratory report.

EXPLORE

Procedure E—Single Muscle Twitch

1. To record a single muscle twitch, set the voltage for a maximal muscle contraction as determined in Procedure D.
2. Set the paper speed at maximum, and with the paper moving, administer a single electrical stimulus to the frog muscle.
3. Repeat this procedure to obtain several recordings of single muscle twitches.
4. Complete Part C of the laboratory report.

EXPLORE

Procedure F—Sustained Contraction

1. To record a sustained muscle contraction, follow these steps:
 - a. Set the stimulator for continuous stimulation.
 - b. Set the voltage for maximal muscle contraction as determined in Procedure D.
 - c. Set the frequency of stimulation at a minimum.
 - d. Set the paper speed at about 0.05 cm/sec.
 - e. With the paper moving, administer electrical stimulation and slowly increase the frequency of stimulation until the muscle sustains a contraction (tetanic contraction or tetanus).
 - f. Continue to stimulate the muscle at the frequency that produces sustained contractions until the muscle fatigues and relaxes.
2. Every 15 seconds for the next several minutes, stimulate the muscle to see how long it takes to recover from the fatigue.
3. Complete Part D of the laboratory report.

Learning Extension

To demonstrate the staircase effect (treppe), obtain a fresh frog gastrocnemius muscle and attach it to the recording system as before. Set the paper control for slow speed, and set the stimulator voltage to produce a maximal muscle contraction. Stimulate the muscle once each second for several seconds. How do you explain the differences in the lengths of successive muscle contractions?

Name _____

Date _____

Section _____

The **A** corresponds to the indicated Learning Outcome(s) listed at the beginning of the laboratory exercise.

Skeletal Muscle Contraction

Part A Assessments



Match the terms in column A with the definitions in column B. Place the letter of your choice in the space provided. **A**

Column A

- a. All-or-none
- b. Latent period
- c. Motor unit
- d. Muscle tone
- e. Myogram
- f. Refractory period
- g. Tetanic contraction (tetanus)
- h. Threshold stimulus
- i. Twitch

Column B

- _____ 1. Minimal intensity of stimulation necessary to trigger a muscle contraction.
- _____ 2. Response of a muscle fiber/motor unit complete contraction if stimulated sufficiently.
- _____ 3. Consists of a single motor neuron and all of the muscle fibers with which the neuron is associated.
- _____ 4. An action of a muscle contraction and immediate relaxation when exposed to a single stimulus.
- _____ 5. The time between stimulation and response
- _____ 6. The time following a muscle contraction during which the muscle remains unresponsive to stimulation
- _____ 7. Forceful, sustained contraction
- _____ 8. Some contraction of muscle fibers when a muscle is at rest
- _____ 9. The recording of the pattern of a muscle contraction

Part B—Threshold Stimulation Assessments



Complete the following:

- 1. What recording system was used for this laboratory exercise? **2** _____
- 2. What was the threshold voltage for stimulation of the frog gastrocnemius muscle? **3** _____
- 3. What voltage produced maximal contraction of this muscle? **4** _____



Critical Thinking Application


Do you think other frog muscles would respond in an identical way to these voltages of stimulation?


Why or why not?

Part C—Single Muscle Twitch Assessments



Complete the following:


1. Fasten a recording of two single muscle twitches in the following space. 





2. On a muscle twitch recording, label the *latent period*, *period of contraction*, and *period of relaxation*, and indicate the time it took for each of these phases to occur. 
3. What differences, if any, do you note in the two myograms of a single muscle twitch? How do you explain these differences? _____


Part D—Sustained Contraction Assessments



Complete the following:

1. Fasten a recording of a sustained contraction in the following space. 

2. On the sustained contraction recording, indicate when the muscle twitches began to combine (summate), and label the period of tetanic contraction and the period of fatigue. 
3. At what frequency of stimulation did tetanic contraction occur?  _____
4. How long did it take for the tetanic muscle to fatigue?  _____
5. Is the length of muscle contraction at the beginning of tetanic contraction the same or different from the length of the single muscle contractions before tetanic contraction occurred? _____ How do you explain this? 

6. How long did it take for the fatigued muscle to become responsive again?  _____